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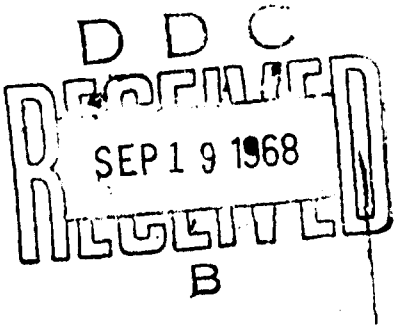

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

THE LUMINESCENCE OF LUMINOL XI

- Yugoslavia -

/Following is a translation of an article by K. Weber and V. Mikulovic of the Establishment for Judicial Medicine and Criminology of the Medical Faculty in Zagreb in the Croatian-language periodical Arhiv higienskoga rada (Archives of Hygiene), No. 10, Zagreb, 1959, pages 101-119./

Use of the Luminol Reaction to Detect Blood

Of all the numerous known chemical method of detecing blood spots for juridical medical purposes, the luminol reaction appears to yield the safest results. The clarity of this reaction is exceptionally great. The correct manner of effecting this reaction to detect blood spots is shown, and the results of the quantitative measurements concerning the influence of various natural substances on the chemiluminescence of luminol are given. Both catalytic and inhibitory effects were noted in the action of these substances. Such influences can alter only slightly the regular course of the luminol reaction in detecting blood stains.

Introduction

In judicial-medical and criminological practice it is often necessary to analyze spots on various objects, and special significance attaches to the detection of blood spots. Various chemical method /1/ /Numbers in brackets refer to

similarly numbered items in Bibliography at end/ for detecting blood in spots are known, such as the benzidine and phenolphtalein test, the reaction with leucomalachite green, etc. These chemical methods of detecting blood are based mostly on the fact that hemoglobin possesses the faculty of peroxidative action, and that this blood coloring, upon execution of the proper chemical test for blood, transfers the oxygen of the hydrogen peroxide to the colorless form of some substrate (benzidine, leuco base, etc.), producing its intensively colored oxidized form. The test yields a positive result for hemoglobin, and hence also for blood, when the expected color appears.

The luminol reaction (chemiluminescence of 3-aminophthalhydrazide) is also based on the above principle /2/ with the difference that in effecting this reaction no color is formed, but the reaction mixture shows a more or less intensive emission of light (chemiluminescence) in the presence of hemoglobin. This light of luminescence can be seen especially well in the dark and has a light blue color. The luminol reaction, in comparison with other similar chemical methods of detecting blood, has a definite advantage, which is pronouncedly manifested in the fact that the luminol reaction has an extremely high brightness, is easy to effect on various objects and also yields a safe result when made with colored test solutions.

The luminol reaction was proposed already in 1937 for the forensic detection of blood spots /3/. It has been used in practice with great success, and the present paper investigates it more closely particularly with regard to the influence of various natural substances on its safety and reliability in detecting traces of blood in spots.

General Remarks about the Luminol Reaction

The chemiluminescence of luminol (3-aminophthalhydrazide) appears when this synthetic compound is oxidized (actually dehydrated) under the influence of active oxygen in alkaline solutions of hydrogen peroxide, sodium perborate, sodium hypochlorite and similar substances. This oxidation of luminol is relatively slow, and the reaction mixture glows only feebly. The hemin proteids -- but also other substances of a certain constitution -- have, however, a pronouncedly catalytic effect on this reaction, and parallel to the increase in the speed of reaction under the influence of the catalyst there is also an increase in the strength of the luminescence of the reaction mixture.

When it is a matter of detecting blood (hemoglobin) by the luminol reaction, hydrogen peroxide is ordinarily used as an oxygen donor, while sodium hydroxide or sodium carbonate may serve as the reaction component forming the alkaline solution. It is important for the luminol reaction with the blood coloring that it is the oxidized form of the hemin proteid that always acts on this reaction, that is, the form with trivalent iron (methemoglobin or hemiglobin), regardless of what form of blood color is added to the reaction mixture. When the reduced form (hemoglobin) is added, the primary process of reaction will be the oxidation of the hemoglobin into hemiglobin, and the thus obtained oxidized form of blood color will act on the luminol reaction as a catalyst. The oxidation of hemoglobin into hemiglobin in alkaline solutions of hydrogen peroxide apparently takes place with greater speed in the presence of sodium hydroxide than in that of sodium carbonate. Hence it is recommended that, in detecting fresh blood, one work with a reagent containing sodium hydroxide, while, in detecting dried blood, which contains the blood color predominantly in hemiglobin form, a reaction mixture with carbonate also yields good results.

Since the luminol reaction also takes place without the presence of a catalyst (blood) in the reaction mixture, the luminol reagent to blood spots is spoiled by standing, so that one should always work with fresh reagent. The solutions of the individual components of the reagent are fairly stable, and their composition is not changed essentially even if they stand for some months. Hence it is recommended that the following be prepared separately: a) a luminol solution in some basic medium, b) a hydrogen-peroxide solution in water, and c) a hydroxide or carbonate solution in water. These solutions are mixed in definite proportions immediately before making the experiments, and the ready luminol reagent thus obtained is usable for two hours at the most. Operation may be recommended with separate solutions of the following composition:

a) luminol 4×10^{-3} M in naOH 5×10^{-2} M
b) hydrogen peroxide 1.76×10^{-1} M
c) NaOH 4×10^{-1} M

The ready luminol reagent is obtained, for example, when five ml each of the above-mentioned initial solutions is added to a quantity of 35 ml of distilled water. For qualitative experiments it suffices to adhere approximately to these relations, while for quantitative measurements one must always work under strictly equal conditions.

The reagent with sodium carbonate may also be prepared according to the above recipe, the sodium hydroxide being replaced in the same molar concentration by sodium carbonate. Of the initial solutions of luminol reagent, the least stable is hydrogen peroxide, since it breaks up constantly at a slow rate into elementary oxygen and water. Hence this solution has more often to be prepared afresh.

A blood spot may also be detected by a luminol reagent containing sodium perborate instead of hydrogen peroxide /4/. Perborate may be regarded as a solid substance containing in each molecule one hydrogen-peroxide molecule: $NaBO_2 \cdot H_2O_2 \cdot 3H_2O$. This solid with active oxygen is very stable, and solutions of a specific concentration of it may be prepared by weighing. The necessary alkaline reaction of the reagent is furnished in this case by the perborate alone, while a solution of tertiary sodium phosphate serves as a solvent for luminol. The following may be recommended as the initial solutions of such a reagent:

a) 0.1% luminol in 1% solution of trisodium phosphate
b) 1% perborate in water.

The ready luminol reagent is obtained when 20 ml of solution a) are mixed with 3 ml of solution b) and 50 ml of water are added. Such a luminol reagent with traces of blood produces an intensive chemiluminescence.

The quantitative experiments reported in this paper were performed with a photoelectric apparatus described in previous publications /5/. The inclination of the galvanometer of this apparatus, designated by G, represents the numerical measure for the relative strength (intensity) of luminescence. When one works under like experimental conditions with the same apparatus, one obtained the same strength of luminescence and the same numerical value for G. The strength of luminescence, even under like experimental conditions, depends, however, on the reaction time (in the figures, t in seconds). The curves showing the dependence of the strength of luminescence on the reaction time, the so-called "intensity curves," have a definite maximum, which regularly appears at the beginning of the action, e.g. in the fifth second of the reaction process. The numerical value of G at this maximum, the so-called "maximum strength of luminescence", is designated by G_m. The maximum speed of the luminol reaction also corresponds to the maximum strength of luminescence, and the maximum speed of reaction is a function -- under otherwise like experimental conditions -- of the concentration of the catalyst (c), that is, in this concrete case, the hemoglobin,

and hence also the blood concentration. In addition, there is also a functional connection between the blood concentration and the total light (L) of luminescence, i.e. the aggregate quantity of emitted light (integral of the intensity curve).

The concentration of hemoglobin in the blood solutions used was determined by measurements of extinction by means of a Pulfrich photometer by the well-known method /67.

Showing the dependance of the maximum strength of luminescence on the concentration of the catalyst or the inhibitor provides a quantitative insight into the action of these substances. In this paper, investigation is made in this manner into various natural substances which might influence the result of the luminol reaction in the practical detection of blood spots.

Results of the Work

A series of intensity curves of luminescence in a carbonate reaction mixture and in the presence of different concentrations of hemoglobin (of fresh blood) is shown in Figure 1. It may be seen that an increase in the hemoglobin concentration regularly augments the maximum strength of luminescence as well as the total light (area beneath the curve). Fundamentally like curves are also obtained in working with other reaction mixtures, and in the presence of a solution of fresh or dried blood.

The dependence of the maximum strength of luminescence on the hemoglobin concentration for a carbonate reaction mixture is shown by the curves in Figure 2. Curve 1 in this figure relates to solutions of fresh blood, that is, to the series of intensity curves in Figure 1, while curve 2 in Figure 2 refers to solutions of dried blood, It may be seen that there is an essential difference in the action of the solutions of fresh or dried blood, in the sense that the dried blood (hemiglobin) catalyzes the luminol reaction in a considerably more pronounced manner than does fresh blood (hemoglobin).

Fundamentally like results are also obtained from a reaction mixture with sodium perborate and luminol in phosphate. Curve 1 in Figure 3 relates to solutions of fresh blood; curve 2, to solutions of blood in water which have stood for 24 hours at room temperature; and curve 3, to solutions of dried blood. When an aqueous solution of blood is left

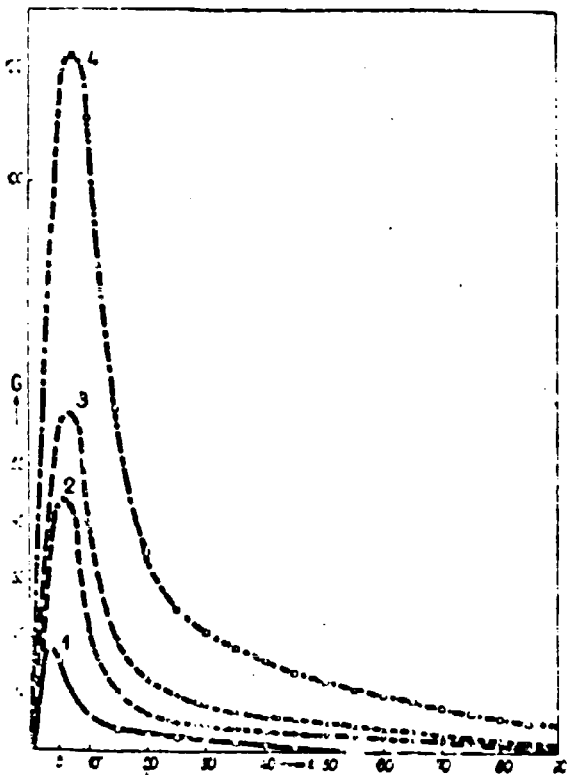

Figure 1. Dependence of the strength of luminescence (G) on the reaction time (t in seconds). Reaction mixture with carbonate. Curve 1. Hemoglobin concentration 1.73 micrograms (γ) in 50 ml of reaction mixture. Curve 2. 5.20 γ. Curve 3. 3.67 γ. Curve 4. 17.33 γ.

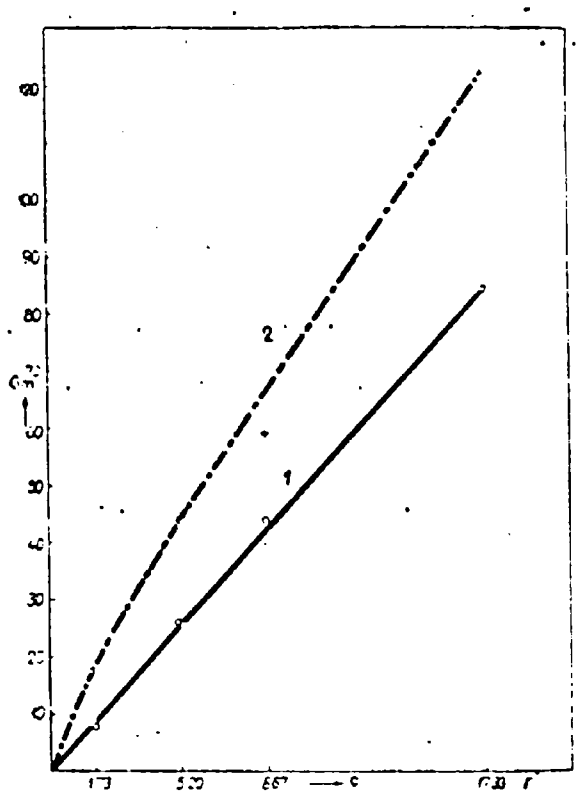

Figure 2. Dependence of the maximum strength of luminescence (Gm) on the hemoglobin concentration (c, micrograms in 50 ml of reaction mixture). Curve 1. solutions of fresh blood. Curve 2. solutions of dried blood. Carbonate reaction mixture.

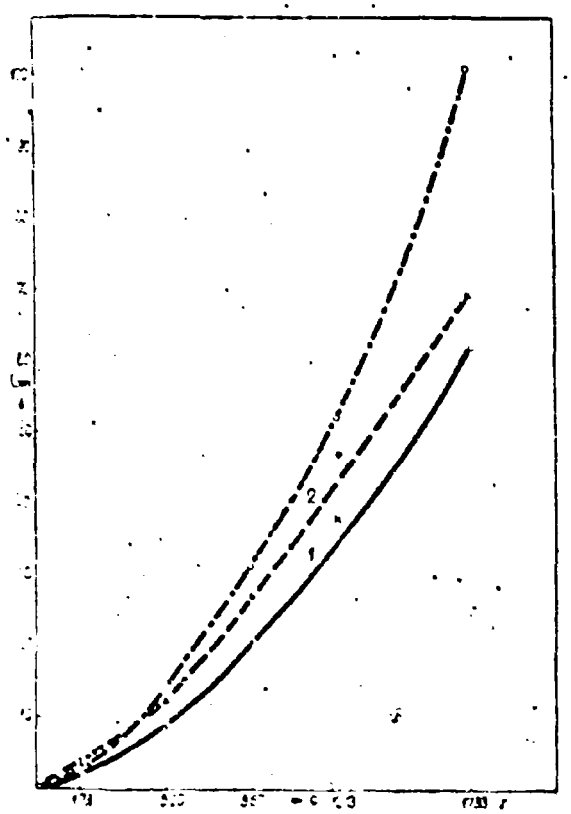

Figure 3. Dependence of the maximum strength of luminescence (Gm) on the hemoglobin concentration. Curve 1. solutions of fresh blood. Curve 2. solutions of fresh blood in water which have stood for 24 hours. Curve 3. solutions of dried blood. Reaction mixture with perborate.

standing, and the blood dries, the hemoglobin gradually changes into hemiglobin, and it may be seen from the course of the above-mentioned curves that in the perborate mixture too the hemiglobin catalyzes the luminol reaction better than does hemoglobin.

When a reaction mixture with sodium hydroxide is used, there is no essential difference, as has already been briefly mentioned, between the action of fresh blood and dried blood. In both cases one obtains the curve of the dependence of the maximum strength of luminescence on the hemoglobin concentration which is shown in Figure 4 (curve 1).

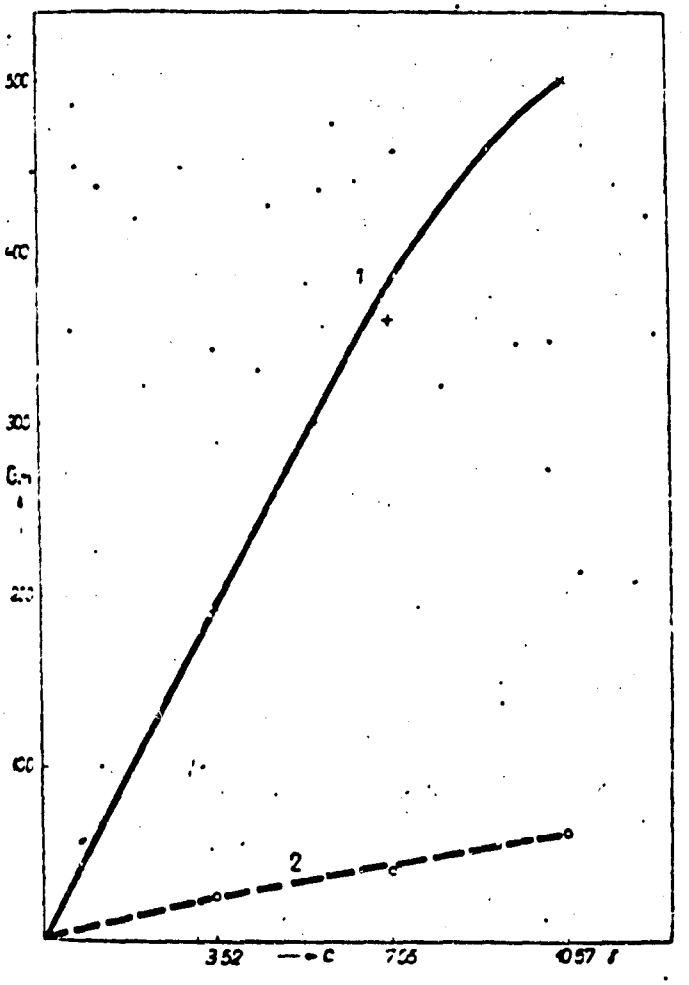

Figure 4. Dependence of the maximum strength of luminescence (Gm) on the hemoglobin concentration (c), and in a reaction mixture with sodium hydroxide. Curve 1. Fresh and dried blood. Curve 2. With the addition of an aqueous extract of human feces.

From the above results of measurements of the maximum strength of luminescence in various reaction mixtures (curves in Figures 2, 3, and 4), it may be objectively ascertained which of the reagents used is best suited for detecting quite small and weak blood spots. From the above data one can compute (read from the curves) that quantity of hemiglobin which, in 50 ml of a given reaction mixture, yields a maximum strength of luminescence of Gm = 50. The following values are obtained:

1.	For $NaOH--H_2O_2$	0.88 microgram
2.	For $Na_2CO_3--H_2O_2$	6.0 micrograms
3.	For $NaBO_2 \cdot H_2O_2 \cdot 3H_2O$	11.5 micrograms

These values are related to one another as 1:6.8:13.0. This means that the detectection of traces of blood by the luminol reaction by the use of a reagent with sodium hydroxide is about seven or eleven times clearer than detection under the same conditions, but with a reagent containing carbonate or perborate. Moreover, in working with a hydroxide it is of secondary importance whether one has to do with fresh or with dried blood spots.

As regards the absolute clearness of the detection of traces of blood, the following may be concluded from the above results of measurements: In a reagent with sodium hydroxide, blood with an approximately normal concentration of hemoglobin (16 g% Hb) in 1:100,000 dilution yields a relative maximum strength of luminescence of Gm = 70, and in a ten times greater dilution Gm = 7 when one ml of blood solution is added to the reaction mixture. Since 10 ml of blood solution may also be added to the reaction mixture under the work conditions shown there, the limit of blood concentration which the luminol reaction can detect can be further lowered. It follows as a final result of these considerations that with the above-mentioned reagent one can detect the presence of blood in an aqueous solution in 1:10,000,000 dilution, and the quantity of hemoglobin causing an inclination of $G_m = 7$ on the galvanometer of the apparatus used will be only 0.16 microgram of Hb. This is indeed an extraodinarily high clarity for a method of detecting traces of blood spots.

Of the importance is the question of the specificity of the detection of traces of blood by the luminol reaction. Since in this reaction it is really a matter of the peroxidative action of hemiglobin, it is clear that any substance with the faculty of peroxidative action can catalyze the luminol reaction. Such natural substances are, above all, the various hemin proteids, animal and plant peroxidases, various

catalases and other enzymes with complexly bound iron /7/ or copper. Of the synthetic substances, various complex compounds of iron, but also of copper /8/, vanadium and ruthenium /9/, act catalytically on the luminol reaction. The number of substances which catalyze the luminol reaction is fairly large, but it may be considered that this reaction, in its practical application, is specific for blood (hemoglobin), since other catalysts act in a considerably less intensive manner, or else they are never in sufficient concentration in the matter ordinarily tested for blood spots. If, however, a matter in which there may be positive catalysts of the luminol reaction is tested for traces of blood, for example, a matter with plant peoxidase (horseradish), the possibility of an erroneous result must be excluded by making a "blind test" with that matter, namely by differential analysis.

In connedtion with these considerations it was of great interest to note what natural substance may essentially affect the strength of the chemiluminescence of luminol in a positive or negative sense, i.e. catalytically or inhibitorily. A series of experiments was therefore performed with aqueous extracts of a substance which appears in connection with blood spots in judicial-medical and criminological practice. The experiments were made with extracts of human, horse, cow and chicken excrement (feces), since the inhibitory action of urine /10/ on the luminol reaction has already been previously noted and investigated in detail. Tests were also made with extracts of horseradish (Armoracia lapathifolia), green paprika (Capsicum annuum), tomato (Solanum hycopersicum) onion (Alium cepa), garlic (Alium sativum), Savoy cabbage (Brassica oleracea varietas sabauda), head cabbage (Brassica oleracea varietas botrytis). It could be presumed that this vegetable matter would contain substances coming into consideration as effectors of the luminol reaction.

In working with aqueous extracts of human, horse, and cow and chicken feces, it has been noted that these extracts contain substances which pronouncedly inhibit the luminol reaction, i.e. extinguish the chemiluminescence of luminol. Figure 4 shows the results of one series of tests with an extract of human feces. The extract was prepared by adding about 8 g of fresh feces and agitated with 200 ml of distilled water, the resulting solution was filtered, and for each test 5 ml of filtrate were used for 45 ml of luminol reaction mixture. The curves in Figure 4 shows the value of the maximum strength of luminescence as a function of the hemoglobin concentration in the absence (curve 1) or presence (curve 2) of

this feces extract. It may be seen that the extract, which obviously contained water-soluble components of the feces in only relatively small concentrations, does strongly inhibit the luminol reaction. Very similar results were also obtained with extracts of horse, cow and chicken feces under approximately like experimental conditions. From the results of these tests it may be concluded that human and animal excrement contains substances which can effectively extinguish the chemiluminescence of luminol, and thus can also have a negative effect on the procedure of detecting traces of blood by the luminol reaction.

Since it has been confirmed several times in the practical application of the luminol reaction in the field that stable manure can also sometimes give a very weak but positive reaction without traces of blood, investigation was made into the ageing process in the aqueous extract of horse and cow feces to test its inhibitory properties with respect to the luminol reaction. Tests were made with aqueous extracts of these feces in the fresh state (directly after extraction), and, in addition, under like experimental conditions after these extracts had been left standing for about four months at room temperature. For the horse- feces extract it was indeed found that there is an essential difference in the action of fresh or old extract on the luminol reaction. The cow feces extract, on the other hand, showed no change in this sense due to ageing. Figure 5 shows the results obtained with the fresh horse feces extract (curve 2) and with the old horse feces extract (curve 3). It may be seen that the fresh extract has an unequivocally inhibitory action, whereas the curve obtained with the old extract takes such a course that a considerably more complicated influence may be assumed. It appears that the ageing in the horse feces extract creates substances which can also have a positive catalytic effect on the luminol reaction, and this catalysis is especially pronounced when the concentration of the other positive catalysts (hemoglobin) is relatively small.

In order to verify the assumption regarding the positively catalytic actior of old horse feces extract, tests were made which yielded the results shown in the curves in Figure 6. In preparing this extract, use was made of 18 g of horse feces, which was agitated with 200 ml of water. These tests were made after the extract had stood at room temperature, for 3½ months. They were made by adding only the extract at first to the luminol reaction mixture, and then measuring the strength of luminescence. The values obtained correspond to the catlytic action of the extract. After 30

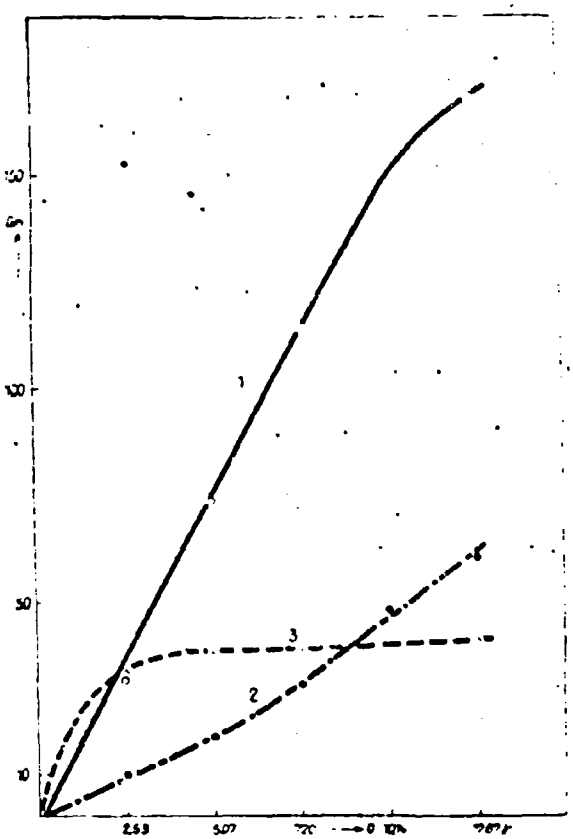

Figure 5. Dependence of the maximum strength of luminescence (Gm) on the hemoglobin concentration (c. γ Hb in 50 ml of reaction mixture with sodium hydroxide). Curve 1. Without feces extract. Curve 2. With fresh extract of horse feces. Curve 3. With old extract of horse feces.

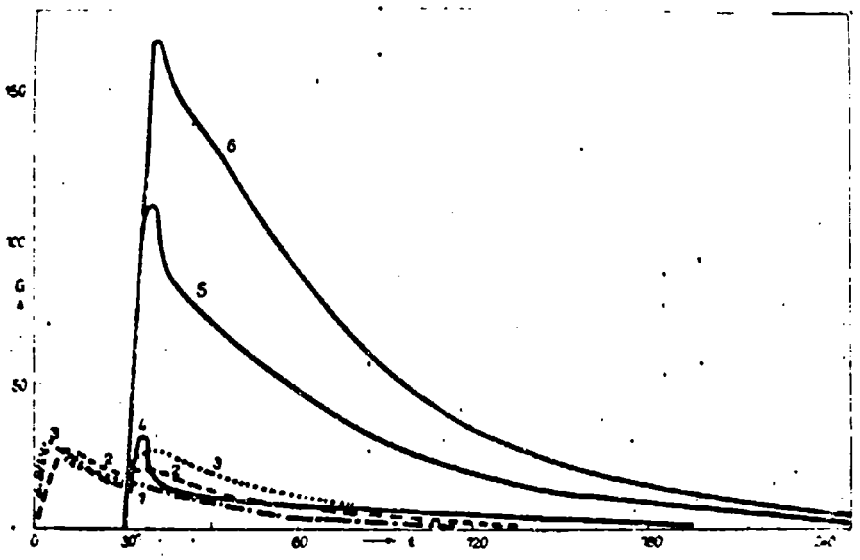

Figure 6. Intensity curves of the luminol reaction in the presence of old extract of horse feces. G = relative strength of luminescence. t = reaction time. Curves 1, 2 and 3 up to t = 30 sec. relate to the catalytic action of the feces extract (2, 6 and 10 ml), and from t - 30 sec. on, to the inhibition occurring when a hemoglobin solution (2.53γ, 7.60γ and 12.65γ in 50 ml of reaction mixture) is added to the same reaction mixture with the same quantity of extract. Curves 4, 5 and 6 relate to reaction mixtures with the same quantities of hemoglobin, but without feces extract.

seconds of reaction time, hemoglobin (blood) solutions were added to the same reaction mixtures, and measurements were then made of the strength of luminescence. The values now obtained corresponded to the inhibited luminol reaction. For comparative purposes, the strengths of the chemiluminescence of the reaction mixtures with the same quantities of hemoglobin were finally measured, but without adding feces extract (curves 4, 5 and 6 in figure 6). According to this, the first parts of curves 1, 2 and 3 in Figure 6 (down to t = 30 seconds) correspond to the catalytic action of old horse feces extract, while the other parts of the same curves, in comparison with curves 4, 5 and 6, show an inhibitory action of the same extracts in the presence of hemoglobin as a catalyst of the luminol reaction.

It may be clearly seen that the catalytic action of the extract is fairly weak, while the inhibitory action is very pronounced. For the practical application of the luminol reaction in detecting blood spots in the possible presence of stable manure, one may conclude the following from the above measurement results; Old stable manure with horse excrement may also contain positive catalysts of the luminol reaction, but the catalytic action of these substances is relatively very weak. The same manure contains, on the other hand, pronounced inhibiters of the luminol reaction. The method of detecting traces of blood in such manure by the luminol reaction thus becomes rather uncertain. If the extract of such manure strongly catalyzes the luminol reaction, it will in any case contain traces of blood. If it exercises, on the other hand, only a weak catalytic action on the chemiluminescence of luminol, it may contain traces of blood, the action of which, however, is inhibited by the other substances present, or else there is no blood in the manure, but only weak positive catalysts.

Very weak positive catalytic influences with respect to the luminol reaction are also inherent in extracts of various kinds of vegetable matter. Figure 7 shows the intensity curves of the luminol reaction relating to the catalytic influence of extracts of horseradish, onion, garlic and green paprika. The extracts were obtained by agitating the following quantities of vegetable matter with 100 ml of distilled water: 1 g of horseradish, 3 g of onion, 2 g of garlic and 8.5 g of paprika. To the reaction mixtures were added extracts in the amount of 10 ml (curves 1, 4 and 5 in figure 7) or 20 ml (curves 2 and 6 in figure 7). It may be seen that the extracts do indeed act on the luminol reaction in a positive catalytic manner, but very weakly. Somewhat stronger is the action of comminuted dry horseradish added directly to the

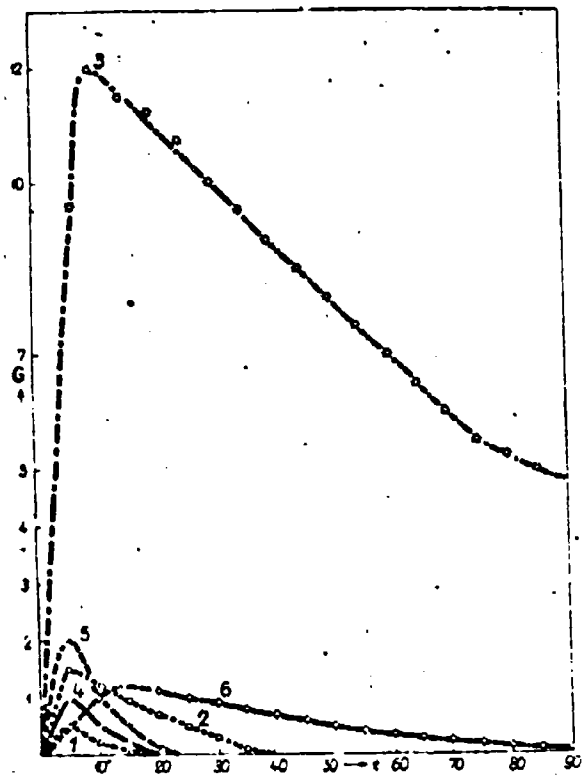

Figure 7. Intensity curves of the luminol reaction catalyzed by various vegetable matters. Curve 1. Horseradish extraction (10 ml). Curve 2. Horseradish extract (20 ml). Curve 3. 0.1 of comminuted dry horseradish was in 50 ml of reaction mixture. Curve 4. Onion extract. Curve 5. Garlic extract. Curve 6. Extract of sharp green paprika. G=relative strength of luminescence. t=reaction time in seconds.

reaction mixture in the amount of 0.1 g. Horseradish contains vegetable peroxidase in rather larger quantity, and it is evident that this hemin proteid caused the catalytic effect noted.

On the other hand it may be assumed that the vegetable matter also contains other substances that can inhibit the luminol reaction. Such a substance would be, first of all, ascorbic acid. Hence, extracts of vegetable matter will ordinarily contain two competing components, a catalytic one and an inhibitory one, and it seems that the latter often predominates. This is why, for example, extract of green paprika, which certainly contains a rather large amount of ascorbic acid, has precisely the least positively catalytic effect.

In the presence of hemoglobin, vegetable extracts ordinarily have a pronounced inhibitory action on the luminol reaction. As an example of such action, Figure 8 shows the dependence of the strength of luminescence on the hemoglobin concentration (micrograms in 50 ml of reaction mixture) in the absence (curve 1) and the presence (curve 2) of 20 ml of aqueous extract of sharp green paprika. It may be seen that the inhibitory action of this extract is indeed very intensive.

The results of identical tests with extracts of Savoy cabbage and head cabbage are shown in Figure 9. It may be seen that under the same experimental conditions the head-cabbage extract inhibits more strongly than does the Savoy-cabbage extract. Fundamentally like results were furthermore obtained with cauliflower extract and tomato juice, with the former showing only a slightly inhibitory effect.

Conclusions

The luminol ~~section~~ may serve very well for the detection of blood spots and traces of blood on various objects in judicial-medical and criminological practice. There are various recipes for preparing the luminol reagent, among which that with sodium hydroxide yields equally intensive chemiluminescence regardless of whether it is a matter of fresh traces of blood (oxyhemoglobin) or dried spots (methemoglobin). The reagents with sodium carbonate or with sodium perborate, on the contrary, yield stronger luminescence with dried blood spots than with solutions of fresh blood under otherwise like experimental conditions. This fact is interpreted by the

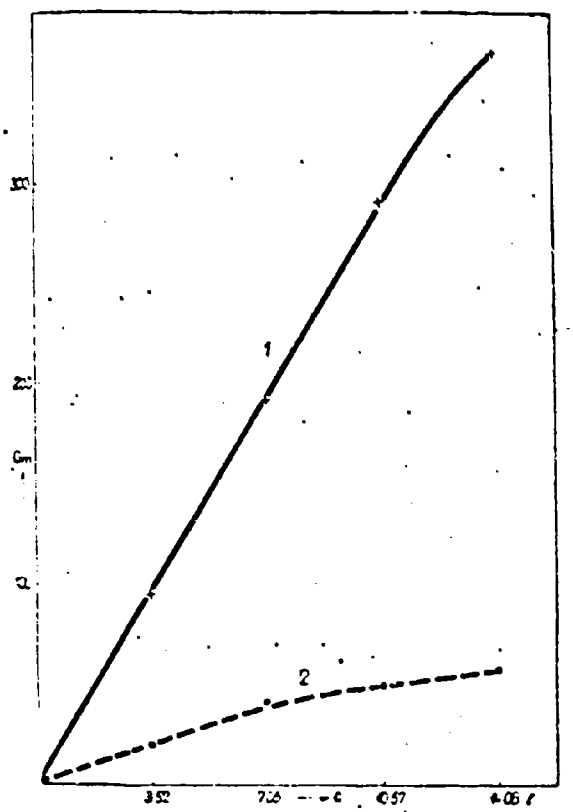

Figure 8. Dependence of the maximum strength of luminescence (Cm) on the hemoglobin concentration (γ in 50 ml of reaction mixture). Curve 1. Without paprika extract. Curve 2. With 20 ml of extract of green paprika (8.5 g in 100 ml of water).

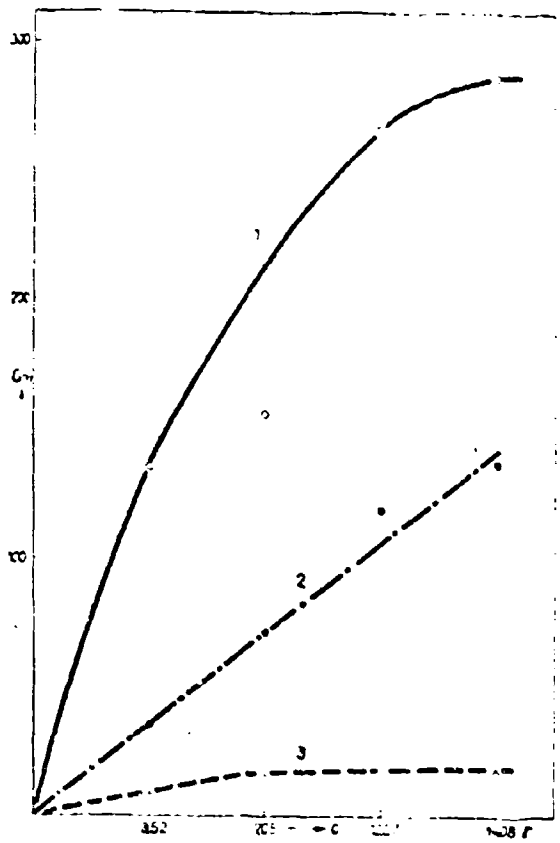

Figure 9. Dependence of the maximum strength of luminescence (Gm) on the hemoglobin concentration (γ in 50 ml of reaction mixture). Curve 1. Without extract. Curve 2. In the presence of 5 ml of Savoy-cabbage extract (6 g in 100 ml of water). Curve 3. In the presence of 5 ml of head-cabbage extract (6 g in 100 ml of water).

assumption that only the oxidized form of blood color (methemoglobin) reacts directly with luminol, and the conversion of hemoglobin into methemoglobin takes place in strongly alkaline solutions in the presence of sodium hydroxide and hydrogen peroxide in a very rapid manner (practically instantaneous in fact), whereas the same reaction in more weakly alkaline solutions, in the presence of carbonate or perborate, is considerably less rapid. Hence it is recommended that in detecting traces of blood use be made of a luminol reagent with sodium hydroxide and hydrogen peroxide.

→ The clearness of the luminol reaction to hemoglobin is extraordinarily great. The quantity of only 0.16 microgram of hemoglobin produce a clearly visible chemiluminescence, the strength of which can be safely measured with a photoelectric apparatus with a selenium photoelement and a lighted mirror galvanometer. The above-mentioned quantity of hemoglobin is found in a volume of 10 ml of blood solution when blood of normal composition is diluted to a proportion of 1:10,000,000.

The specificity of the method of detecting traces of blood by the luminol reaction is determined precisely by the high clarity of the reaction of luminol with hemoglobin. Among the natural substances, solely other hemin proteids, particularly vegetable peroxidase, produce such reactions with luminol. Hence, in effecting this reaction with various kinds of matter, one must exclude the presence of such natural catalysts of the luminol reaction.

Aqueous extracts of human, horse and cow excrement (feces) contain substances which pronouncedly inhibit the luminol reaction catalyzed by traces of blood (hemoglobin). An old horse feces extract contains, in addition, substances which act as positive catalysts of the luminol reaction. This positively catalytic action is, however, extraordinarily weak. Stable manure may, then, yield a very weak positive reaction with luminol even without the presence of traces of blood, and even in the presence of traces of blood the luminol reaction on this matter need not be strong. Hence it is rather difficult to prove with certainty the presence of traces of blood in stable manure.

Aqueous extracts of various kinds of vegetable matter have a preponderantly inhibitory action on the luminol action, i.e. they pronouncedly extinguish the chemiluminescence of luminol. This inhibitory action is ascribed to the component parts of plants with reductive properties. Ascorbic acid in

particular is such a substance. However, there are also positive catalysts of the luminol reaction in vegetable matter. The vegetable peroxidase in horseradish is, for instance, such a positive catalyst. But these positive catalytic effects are always very weak due to the influence of the vegetable matter, so that the inhibitory action ordinarily predominates.

On the basis of the results of the measurements made it may be stated in general that in the practical detection of blood spots by the luminol reaction one must by all means preclude the possible inhibitory influences of substances from animal excrement or vegetable matter. The positive catalytic effects of foreign substances will, on the contrary, play no significant role. If one proceeds further in the evaluation of the results of the luminol reaction in such a way as to regard only strong luminescence as a positive proof of the presence of traces of blood, this reaction will represent a reliable working method.

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